

**MANUSCRIPT 4**

**SUPPLEMENTAL LABORATORY  
TESTING  
AND QUALITY ASSURANCE/  
QUALITY CONTROL**

**CITY OF LINCOLN, NEBRASKA  
SALT CREEK WATER QUALITY STUDIES**

**April 3, 2000  
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# SUPPLEMENTAL LABORATORY TESTING AND QUALITY ASSURANCE/ QUALITY CONTROL

## Key Findings

The following are Key Findings from the Supplemental Laboratory Testing and Quality Assurance/Quality Control program which highlight the main points and conclusions. Extensive detail on the program, final results and final conclusions are also presented within this Manuscript.

- In direct support of the *in-situ* testing program, laboratory testing was conducted to ensure that the data quality would be acceptable in a research/regulatory program of this type.
- Laboratory work included: (1) Extensive toxicity testing to determine the health of the test species and the sensitivity of different life stages to ammonia; (2) A comprehensive evaluation of key water quality parameters (e.g., ammonia, chloride, pH, DO); and (3) Analyses of pesticides and herbicides and Whole Effluent Toxicity (WET) testing to help evaluate whether other toxicants might have affected the study.
- The results of the laboratory work showed no anomalous results and strengthen the scientific defensibility of the *in-situ* program results.

## 1.0 INTRODUCTION

In direct support of the in-situ program, extensive efforts were conducted to ensure that data quality would be acceptable in a research/ regulatory program of this type. These laboratory efforts included:

- salinity tolerance testing of 30- to 45-day old fathead minnows and channel catfish;
- salinity tolerance testing of < 24-hour old fathead minnow larvae;
- test organism age study to quantify the sensitivity of < 24-hour old fathead minnows versus 30- to 45-day old fathead minnows to ammonia;
- examination of the quality of test organisms using reference toxicant testing;
- parallel chronic [WET testing](#) (during *in situ* program);
- an *in situ* salinity tolerance study using a series of control stations in several salinity zones (e.g., 1, 2, and 4 ppt, located upstream of the Theresa Street WWTP); and
- water quality monitoring of pesticides and herbicides at three instream locations at three different times during the in-situ program

The results of these studies are briefly summarized below, and detailed separately in later sections of this Manuscript. Additional details for each of these studies are contained in individual laboratory reports generated for each test.

#### Salinity tolerance of juvenile fathead minnows and channel catfish ([Section 2.1](#))

- For *juvenile* fathead minnows, there was no effect on survival from 30-day exposures to salinities ranging from 0-10 ppt. After 15 days exposure, there was an effect on growth at salinities of 4, 6 and 8 ppt.
- For *juvenile* channel catfish, there was no effect on survival or growth at salinity concentrations as high as 10 ppt after a 30-day exposure.

#### Larval fathead minnow acute sensitivity to salinity ([Section 2.2](#))

- Simulated saline Salt Creek water was acutely toxic to larval fathead minnows (<24-hr old). The LC50 value was calculated to be 3.8 ppt.
- There was 70 percent survival in a 3.4 ppt sample of ambient Salt Creek water.

#### Reference toxicant testing of laboratory organisms ([Section 2.3](#))

- Acute toxicity tests using a reference toxicant (sodium dodecyl sulfate) indicated that the test organisms were of acceptable quality.

#### Whole Effluent Toxicity (WET) testing during the in-situ program ([Section 2.4](#))

- WET tests were conducted on wastewaters from both the Theresa Street and Northeast WWTP's on two occasions (September and October, 1999). Studies included acute and chronic testing as well as ammonia removal and ammonia spiking experiments.

#### Chronic toxicity testing using ambient Little Salt Creek water ([Section 2.5](#))

- There was zero percent survival of *C. dubia* in undiluted Little Salt Creek water at a conductivity of ~10,000  $\mu\text{S}/\text{cm}$  ( $\approx 6.5$  ppt), and also at 50 percent creek water (~3.2 ppt) during the 3-brood chronic test.
- Dilutions of the Little Salt Creek ambient water sample (~6.5 ppt) did not adversely affect larval fathead minnow survival or growth over the 7-day exposure period

#### Fathead minnow chronic testing to examine age sensitivity to ammonia ([Section 2.6](#))

- 30-day chronic tests with *larval* fathead minnows showed no effect on survival or growth at the highest measured exposure concentration (11.6 mg-N/L).

- 30-day chronic tests with 30-45 day old *juvenile* fathead minnows showed no effect on survival, but a statistically significant effect on growth at the highest measured concentration (21 mg-N/L).

## **2.0 TOXICOLOGY LABORATORY STUDY RESULTS**

### **2.1 Salinity Tolerance of Juvenile Fathead Minnows and Channel Catfish**

Salinity tolerance and growth rate testing of juvenile fathead minnow, *Pimephales promelas* and channel catfish, *Ictalurus punctatus* was conducted at EA's Ecotoxicology Laboratory (Sparks, Maryland) from 2 September to 2 October, 1999. Detailed reporting of these studies including materials and methods, data analysis and raw data sheets are presented in EA Report #3305.

#### **2.1.1 Study Objectives**

Separate salinity/growth studies were conducted with juvenile (~45 days old) fathead minnow (*Pimephales promelas*) and juvenile catfish (*Ictalurus punctatus*) over 15 to 30 day exposure periods. The studies were conducted to determine if salinity, at levels encountered in Salt Creek, has an inhibitory effect on the growth rate of juvenile fathead minnows and channel catfish. If an inhibitory effect on growth was encountered for either fish species, a secondary objective was to develop a "salinity correction factor" that could potentially be applied to the growth data generated in the Salt Creek in-situ caged fish growth data. The saline water used to conduct the studies had an ionic makeup that was formulated to simulate the ionic composition of Salt Creek ambient water. Two fathead minnow salinity studies with exposure durations of 15 and 30 days were performed, along side a single channel catfish 30-day duration salinity study.

#### **2.1.2 Test Results**

The results of the fathead minnow 15-day study that evaluated the effect of salinity (2, 4, 6 and 8 parts per thousand; ppt) on the growth of juvenile fish are presented in [Table 4-1](#). The table presents the *P. promelas* survival data at 7 and 15 days, and also the mean dry weight, at the end of 15 days. Survival was very high at the end of 7 and 15 days. There was a minimum of 97 percent survival in the salinity treatments, the control survival was 100 percent, and none of the salinity treatments were significantly different from the control based on survival.

Statistical analyses of the dry weight data indicated that over a 15 day exposure period, fathead minnow growth was significantly ( $p=0.05$ ) less than the control in salinity treatments of 4, 6, and 8 ppt salinity. The dry weight data exhibited a good dose response to the salinity treatments. The mean dry weight of 16 mg/organism in the control was not significantly different than the 15.2 mg/organism in the 2 ppt treatment, but was significantly greater than the 13.5, 13.5 and 12.1 mg/organism in the 4, 6 and 8 ppt treatments, respectively. The salinity LOEC was 4 ppt, the NOEC was 2 ppt, and the resulting ChV was 2.8 ppt. The 20 percent inhibition concentration (IC20) for this test was calculated to be 7 ppt salinity.

The results of the 30-day fathead minnow salinity/growth study are presented in [Table 4-2](#). There were originally 4 replicate test chambers for each salinity treatment in the fathead minnow

growth study. Two replicates were terminated for the 15-day study and the remaining two replicates continued on for the 30-day study. On day 18 of the 30 day exposure, an unexplained toxic batch of synthetic creek water was encountered, which resulted in significant fathead minnow mortality in the 6 and 8 ppt salinity treatments on the day 19 headcounts. The day-15 survival in the 30-day study is listed in [Table 4-2](#), and at day 15, the minimum mean survival in the test treatments was 97 percent. Between Day 18 and Day 19 there was very high mortality in the 6 and 8 ppt salinities (90 and 100 percent mortality, respectively) thus eliminating those treatments from the 30 day study.

Based on the 30-day fathead minnow survival and biomass data, the 2 and 4 ppt treatments were not significantly different from the control after 30 days of exposure. The salinity NOEC for the 30-day data was 4 ppt, while the LOEC and ChV were 6 ppt and 4.9 ppt, respectively. Because of the unexplained mortality in 6 ppt and 8 ppt, those treatments were deleted from the IC20 determination. The resulting IC20 value was calculated to be >4.0 ppt salinity.

[Table 4-3](#) presents the results of the 30-day channel catfish salinity/growth study. Out of the entire test, there was only a single mortality, which occurred in the 8 ppt treatment on day 19 of the 30-day test period; thus salinity (up to 10 ppt) had no effect on catfish survival. With respect to the catfish dry weight biomass data, the 2 ppt salinity treatment (which was considered to be the control for this species) has a mean biomass of 199.9 mg/organism. The next two higher salinities of 4 ppt and 6 ppt had increased (compared to the control) biomass values of 218.7 and 214.9 mg/organism respectively. While the two highest test salinities of 8 ppt and 10 ppt had slightly lower biomass (187.0 and 185.6 mg/organism) than the control, those biomass values were not significantly less than the control ( $p=0.05$ ). The salinity NOEC for this study was 10 ppt, and the LOEC and ChV were both >10 ppt. The IC20 for the 30-day catfish study was >10 ppt.

### **2.1.3 Discussion**

When exposed to salinities ranging from 0-10 ppt, fathead minnow survival was not affected after 15- or 30-day exposures. During the first 15-days of exposure, growth (mean biomass) was not different from controls at the lowest salinity exposure concentration (2 ppt), but significantly lower than controls at 4, 6 and 8 ppt. In the 30-day exposures, the 2 and 4 ppt salinity treatments were not different from control. At the two higher salinities (6 and 8 ppt), however, mortality from a toxic batch of synthetic creek water precludes the ability to make comparisons.

## **2.2 Larval Fathead Minnow Acute Sensitivity to Salinity**

A 96-hour acute renewal toxicity test was conducted with less than 24 hours old fathead minnow (*P. promelas*) to determine their acute sensitivity to a simulated Salt Creek synthetic saltwater. The test was initiated on 19 October 1999 at EA's Ecotoxicology Laboratory (Sparks, Maryland) and detailed descriptions of test methods, results, raw data, etc. are presented in EA report #3305. The objective of the study was to determine the maximum salinity of a simulated Salt Creek saltwater that the larval fathead minnows could tolerate over a 96-hour exposure period. Included in the test was a 3.4 ppt ambient Salt Creek sample collected on 18 October 1999 which was tested at 100 percent only.

### **2.2.1 Test Results**

The results of the larval fathead minnow acute toxicity test are presented in [Table 4-4](#). The 96-hour percent survivals in test salinities of 1.8, 2.8, 4.2, 6.5, and 10 ppt were 85, 60, 50, 55, and 10 percent, respectively. Control survival at 96 hours was 100 percent. The resulting 96-hour LC50 was 3.8 ppt, and the 95 percent confidence interval was 3.0 - 5.0 ppt simulated Salt Creek saltwater. Survival in the 3.4 ppt ambient Salt Creek sample was 70 percent.

### **2.2.2 Discussion**

The results of this test indicate that larval fathead minnow (<24 hours old) potentially are not an appropriate life stage of fish for testing ambient waters with salinities which are normally encountered in Salt Creek. Acceptable control survival of  $\geq 90$  percent would most likely not be attained due to salinity alone, making the toxicity contribution of other constituents (e.g. ammonia) difficult to assess.

## **2.3 Examination of the Quality of In-situ Test Organisms using Reference Toxicant Testing**

In conformance with EA's quality assurance/quality control program, reference toxicant tests were performed on the lots of fathead minnows (*Pimephales promelas*), and channel catfish (*Ictalurus punctatus*), which were used in the Salt Creek in-situ caged fish studies. Channel catfish (Lot # IP- 005) and fathead minnow (Lot # FH- 236) were received at EA on 8/18/99 and 9/15/99, respectively. The acute toxicity tests were initiated on 09 September (channel catfish) and on 16 September 1999 (fathead minnow). The results of the testing are included in EA Report #3305.

### **2.3.1 Test Objective**

The objective of the tests was to determine the 24-hour (*P. promelas*) and 48-hour (*I. punctatus*) acute response of the organisms to the reference toxicant, to provide an indication of the health/quality of the test populations. The 24-hour *P. promelas* test was conducted with a graded concentration series of sodium dodecyl sulfate (SDS) and a dilution water control. The 48-hour *I. punctatus* test also utilized SDS as the reference toxicant. The results were compared to established control chart limits set by EA, or were compared directly to previous test results if an insufficient database existed to calculate acceptable control chart limits.

### **2.3.2 Test Results**

The results of reference toxicant test conducted on a sub-group of the channel catfish population that was used in the Salt Creek *in-situ* caged fish study are presented in [Table 4-5](#). The test consisted of a graded concentration series of sodium dodecyl sulfate (SDS). The nominal test concentrations were 60, 40, 20, 10 and 5 mg/L SDS and dilution water control. There was zero percent survival at 60 and 40 mg/L SDS, 90 percent survival at 20 mg/L, and 100 percent survival in the control and 5 and 10 mg/L SDS. The resulting 48-hour LC50 was 26.3 mg/L SDS. An insufficient *I. punctatus* database exists for the calculation of reportable control chart limits, however the present value is in relatively good agreement with previous 48-hour SDS LC50 values (10.6, 14.1 and 20.0 mg/L SDS).

[Table 4-6](#) presents the results of the SDS reference toxicant test with juvenile fathead minnows. The nominal SDS test concentration series was identical to the channel catfish series, and the results were also nearly identical. The resulting 24-hour LC50 was 28.3 mg/L SDS. EA does not presently have active control chart acceptability limits for juvenile fathead minnows, because that age is no longer utilized in NPDES testing programs. The current LC50 falls within the historic control chart acceptability limits for juvenile fathead minnow, which were 11.2 – 45.2 mg/L SDS.

## **2.4 Parallel Whole Effluent Toxicity (WET) Testing During the In-Situ Testing Program**

During the timeframe that the Salt Creek in-situ caged fish study was being conducted, [whole effluent toxicity](#) (WET) testing was conducted on wastewaters from the Theresa Street and Northeast WWTP's on two separate occasions (September and October, 1999). The WET

studies consisted of acute and chronic toxicity tests with fathead minnow (*P. promelas*) and *Ceriodaphnia dubia*. In addition to evaluating the toxicity of the whole effluents, tests were conducted on ammonia stripped samples of the wastewaters as well as aliquots of ammonia stripped samples with ammonia added back to original levels. Note that the City's Northeast WWTP was being run during this period at substantially higher than normal ammonia concentrations so that the in-situ program would have appropriate in-stream exposures.

#### **2.4.1 Study Objective**

One objective of the WET studies was to document the acute and chronic toxicity of these wastewater treatment plant effluents on two separate occasions during the Salt Creek in-situ caged fish study. A second objective was to attempt to quantify the contribution of ammonia to the two wastewaters' acute and chronic toxicity.

#### **2.4.2 Test Results**

The September WET tests were conducted on Theresa Street WWTP and Northeast WWTP effluent samples collected the week of 26 September 1999. The acute toxicity tests were conducted on effluent composite samples collected from 26-27 September, while the chronic toxicity tests were initiated with the same samples, and were renewed with composite samples collected from 28-29 September; and from 30 September – 01 October 1999. A detailed description of the test methods and results for the acute and chronic toxicity tests can be found in EA Report numbers 3214 and 3215, respectively.

The results of the 24-hour acute toxicity tests conducted on the two WWTP effluents are presented in [Table 4-7](#). For the Theresa Street WWTP effluent, the 24-hour *Ceriodaphnia dubia* and *Pimephales promelas* LC50s were 37.0 and 49.2 percent effluent, respectively. Ammonia stripping of the Theresa Street effluent removed the effluent's acute toxicity to *P. promelas*, but not to *C. dubia*. The Northeast WWTP effluent was not acutely toxic to *C. dubia*, but produced a 24-hour LC50 of 83.3 percent with *P. promelas*. Similar to the Theresa Street WWTP wastewater, ammonia stripping of the effluent sample removed the acute toxicity to *P. promelas*.

The results of the September *C. dubia* and *P. promelas* chronic toxicity test are presented in [Table 4-8](#). The Theresa Street WWTP wastewater elicited very similar toxicity responses from the two test species. For the Theresa Street effluent, the *C. dubia* and *P. promelas* NOEC, LOEC and ChV values were 25, 50 and 35.4 percent effluent respectively. The IC25s for *C. dubia* and *P. promelas* were also nearly identical (31.3 and 30.7 percent effluent respectively). Ammonia stripping of the Theresa Street WWTP wastewater removed a majority of the chronic toxicity to *C. dubia* (NOEC = 80 percent), whereas all the measurable chronic toxicity to *P. promelas* was removed (NOEC = 100 percent).

The results of the September 1999 chronic toxicity tests on the Northeast WWTP wastewater samples are presented in [Table 4-9](#). The Northeast whole effluent samples were chronically toxic to *C. dubia* and *P. promelas*. For each test species, the NOEC's were 25 percent effluent, the LOEC's were 50 percent effluent, and the ChVs were both 35.4 percent effluent. The IC25s were 36.4 percent for *C. dubia*, and 55.1 percent for *P. promelas*. For the Northeast WWTP



effluent, ammonia stripping removed all the chronic toxicity to *C. dubia* and *P. promelas* (IC25s >100 percent effluent).

The October 1999 acute and chronic WET tests were conducted on Theresa Street and Northeast WWTP effluent composite samples collected during the week of 10 October 1999. The first effluent composite sample for the test initiations was collected on 10-11 October; and the second and third samples, for the chronic toxicity test renewals, were collected on 12-13 and 14-15 October 1999 respectively. With respect to acute toxicity ([Table 4-10](#)), neither WWTP wastewater was toxic to *C. dubia* (24-hour LC50s >100 percent effluent). The Theresa Street WWTP effluent was slightly more acutely toxic to *P. promelas* than the Northeast effluent as evidenced by 24-hour LC50s of 59.0 and 65.2 percent effluent respectively. Ammonia stripping removed the acute toxicity to *P. promelas* for both WWTP effluents (LC50s >100 percent).

The results of the October 1999 chronic toxicity tests conducted on Theresa Street WWTP effluent composite samples are presented in [Table 4-11](#). The Theresa Street wastewater was more chronically toxic to *P. promelas* than *C. dubia*. For both species, survival was adversely effected at 50, 80 and 100 percent effluent. The 25 percent effluent concentration adversely affected *P. promelas* growth, but did not affect *C. dubia* survival or reproduction. The NOECs for *P. promelas* and *C. dubia* were 12.5 and 25 percent respectively, while the IC25s for the two species were similar at 31.2 and 33.1 percent respectively.

The Northeast WWTP wastewater had an adverse impact on *C. dubia* reproduction at 80 and 100 percent effluent ([Table 4-12](#)), and had an effect on *P. promelas* survival at 50, 80 and 100 percent. The *C. dubia* and *P. promelas* NOECs were 50 and 25 percent respectively, however the IC25s were similar at 47.8 and 48.5 percent effluent respectively. Ammonia stripping of the two WWTP effluents removed the chronic toxicity to *P. promelas* but only reduced (did not completely remove) the chronic effluent toxicity to *C. dubia*.

## **2.5 Short-term Chronic Toxicity Tests with Ambient Little Salt Creek Water**

A *Ceriodaphnia dubia* 3-brood survival and reproduction test, and a *P. promelas* 7-day larval survival and growth test were conducted on an ambient sample of Little Salt Creek water collected on 14 December 1999. Detailed descriptions of the materials and methods, results and data analyses are presented in EA Report #3298.

### **2.5.1 Study Objectives**

One objective of this study was to evaluate the toxicity of ambient creek water during a period when the conductivity was relatively high (approximately 10,000  $\mu\text{S}/\text{cm}$ ; approximately 6.5 ppt salinity). During the proposed study period, the conductivity of Salt Creek was substantially more dilute, therefore Little Salt Creek was selected for sampling because the conductivity was at the desired level (9,980  $\mu\text{S}/\text{cm}$ ), and the ionic constituency of Little Salt Creek is nearly identical to the ionic makeup of Salt Creek. Another objective was to determine if standard freshwater NPDES chronic toxicity test methods are appropriate for evaluating the toxicity of saline ambient waters.

### 2.5.2 Test Results

[Table 4-13](#) presents the results of the *C. dubia* 3-brood survival and reproduction test with the Little Salt Creek ambient water sample. There was zero percent survival of *C. dubia* in the 100 and 50 percent concentrations at test termination on Day 6. Young production in the 25 percent concentration (18.5 young per female) was significantly lower than the 22.7 young per female in the control. Young production in the 12.5 and 6.25 percent concentrations was not significantly different than the control. The *C. dubia* NOEC for this test was 12.5 percent Little Salt Creek water, the chronic value (ChV) was 17.7 percent, and the IC25 was 27.0 percent creek water.

The *P. promelas* 7-day larval survival and growth test results are summarized in [Table 4-14](#). The Little Salt Creek ambient water sample did not have an adverse effect on *P. promelas* survival or growth at any test concentration over the 7-day test period. The 88 percent survival at 100 percent Little Salt Creek was not statistically different than the 100 percent control survival. The mean dry weights (growth) in the four highest test concentrations of 12.5 through 100 percent sample (0.726 – 0.832 mg/organism) were all greater than the control mean dry weight of 0.665 mg/organism. The *P. promelas* NOEC was 100 percent, and the ChV and IC25 values were both >100 percent Little Salt Creek sample.

## 2.6 Fathead Minnow Age Sensitivity to Ammonia Toxicity

Chronic toxicity tests were conducted in the laboratory to quantify the sensitivity of two early life stages of fathead minnow (<24 hour old, and 30-45 day old) to ammonia at a salinity of two parts per thousand (ppt).

### 2.6.1 Study Objectives

Separate chronic ammonia toxicity tests were conducted on larval (<24 hours old) fathead minnow, and on juvenile (30-35 day old) fathead minnows. The goal of the study was to quantify the differential sensitivity (if any) of the two life stages to ammonia at a mid-range salinity normally encountered in Salt Creek. The results of the study could potentially be used to determine an “age correction factor” for ammonia to be applied to the results of the Salt Creek in-situ caged fish study.

### 2.6.2 Test Results

The results of the 30-day chronic ammonia toxicity test using larval (<24 hours old) fathead minnows are presented in [Table 4-13](#). The test results presented include the 7-day and 30-day survivals, as well as the 30-day mean dry weight, and the 30 day mean biomass data. The nominal ammonia test concentration series for the larval test was control, 0.81, 1.8, 3.7, 7.5 and 15.5 mg-N/L. The selected concentration series was based on ammonia chronic toxicity values in the literature (U.S. EPA 1999). The results of ammonia analyses performed on new and old test solutions are presented in [Table 4-14](#). It was observed that while a number of the measured ammonia concentrations were at the nominal target ammonia levels, in general; the mean measured ammonia concentrations in the larval fathead minnow test were approximately 75% of the target nominal levels.

The 7-day larval survival rates were  $\geq 90$  percent in all test concentrations and control; and after 30 days of exposure, there was 80 percent or greater survival in all of the test treatments. The control survival after 30 days was 88 percent, and was not significantly different than any of the ammonia test treatment results. In fact, the highest percent survival after 30 days of exposure was in the highest (15.5 mg/L) nominal ammonia concentration.

The fathead minnow growth data, expressed both as mean dry weight, and as biomass also indicated that at the concentrations tested, ammonia did not elicit a significant adverse impact on fathead minnow growth over the 30-day test period. The highest mean dry weights were observed in the intermediate (1.8 and 3.7 mg-N/L) ammonia test concentrations. In evaluating the measured water quality parameters, and more specifically the test pHs, the highest measured pH values occurred in the control and low concentration new solutions, and the highest ammonia test concentration had more stable, and slightly lower pH values. With respect to survival and growth, the 30 day NOEC for this test was 11.65 mg-N/L (measured) and the 30-day LOEC, ChV and IC20 were all  $>11.65$  mg-N/L  $\text{NH}_3\text{-N}$ .

[Table 4-15](#) presents the results of the 30-day ammonia chronic toxicity test with juvenile fathead minnows. The nominal ammonia concentration series for this test dropped the lowest treatment from the larval test (0.87 mg-N/L) and added a 32 mg-N/L concentration at the high end of the concentration series, thus the juvenile fathead minnow test series was 1.8, 3.7, 7.5, 15.5 and 32 mg-N/L. After 30 days of exposure, juvenile fathead minnow survival rates were high ( $\geq 87$  percent) in all ammonia test concentrations. The control survival of 97 percent was not significantly different from any of the ammonia treatments indicating that survival was not adversely affected over the 30-day test period.

Analysis of the biomass data detected a significant effect on growth at the highest nominal ammonia concentration of 32 mg-N/L. The mean biomass of the control was 29.15 mg/organism, which was significantly ( $P=0.05$ ) greater than the 32 mg-N/L mean biomass of 20.99 mg/organism. The NOEC for the juvenile test was 15.5 mg-N/L, and the LOEC was 32 mg-N/L. The ChV and the IC20 were 27.3 and 25.2 mg-N/L respectively. The measured ammonia concentrations for the juvenile fathead minnow test were again approximately 75% of nominal except for the highest nominal concentration of 32 mg/L which had mean ammonia concentrations of approximately 66 percent nominal ([Table 4-16](#)).

### **3.0 AMBIENT MONITORING OF SALT CREEK FOR PESTICIDES AND HERBICIDES**

During the *in situ* testing, limited pesticide and herbicide sampling was conducted to evaluate other significant factors that could potentially affect the *in situ* program results. Pesticide and herbicide samples were collected at three times during the 30-day in-situ program at three locations: (1) upstream of the Theresa Street WWTP – Location C; (2) just above the Northeast WWTP – Location D; and (3) below the Northeast WWTP - Location I.

Samples consisted of five individual grab samples taken across the stream channel (equal distances apart). The grab samples were then immediately composited and shipped on ice in

coolers by overnight carrier to Severn Trent Laboratories (Sparks, MD) and personally delivered by EA staff to Midwest Laboratories in Omaha, Nebraska.

- Dates of Collection -  
(1) 24 September 1999, (2) 5 October 1999, (3) 20 October 1999
- Severn Trent Laboratories Analyses
  - (1) Chlorinated Pesticides/PCBs by GC (Method SW-846 Method 8081A/8082)
 

Alpha-BHC	gamma-BHC	Heptachlor
Aldrin	beta-BHC	Delta-BHC
Heptachlor Epoxide	Endosulfan I	Gamma-Chlordane
Alpha-Chlordane	4,4'-DDE	Dieldrin
Endrin	Endosulfan II	4,4'-DDD
4,4'-DDT	Endrin Aldehyde	Endosulfan Sulfate
Methoxychlor	Endrin Keton	Toxaphene
Arochlor(s)-1016,1221,1232,1242,1248,1254,1260		
  - (2) Organophosphorus Pesticides by GC (SW-846 Method 8141)
 

Dichlorvos	Mevinphos	Demeton
Ethoprop	Naled	Phorate
Diazinon	Disulfoton	Methyl parathion
Ronnel	Malathion	Fenthion
Chlorpyrifos	Trichloronate	Merphos
Merphos DEF	Stirophos	Tokuthion
Fensulfotion	Bolstar	Azinphos methyl
Coumaphos		
  - (3) Herbicides by GC (SW-8468 Method 8151)
 

Dalapon	Dicamba	MCPP
MCPA	Dichloroporp	2,4-D
PCP	2,4,5,-TP	2,4,5,-T
Dinoseb	2,4-DB	Picloram
- Midwest Laboratories Analyses Herbicides/Pesticides (Method EPA 507 Modified)
 

Acetochlor	Alachlor(Lasso)	Atrazine (Aatrex)
Benfluralin (Balan)	Butachlor	Butylate (Sutan)
Chlorpyrifos (Dursban, Lorsban)		Dimethenamid
EPTC (Eptam, Eradicane)	Cyanazine (Bladex)	Ethalfuralin (Sonalan)
Fonofos (Dyfonate)	Metolachlor (Dual)	Metribuzin (sencor)
Pendimethalin (Prowl)	Phorate (Thimet)	Prometon (Pramitol)
Propachlor (Ramrod)	Propazine (Milogard)	Simazine (Princep)
Terbofos (Counter)	Trallate (Far-go)	Trifluralin (Treflan)
Deethylatrazine	Deisopropylatrazine	Ametryn
Prometryn		

For this large suite of herbicide and pesticide analytes, few were detected in the six Salt Creek ambient water samples. Those that were detected are presented in [Table 4-17](#), and the concentrations are very low. No chemicals were “detected” and “unqualified” which exceed U.S. EPA’s criteria for the protection of aquatic life.

#### **4.0 OVERALL CONCLUSIONS FROM THE LABORATORY PROGRAM**

Extensive laboratory studies were conducted to help support the in-situ program. Overall conclusions are as follows:

- Growth of fathead minnow *juveniles* appears to be slightly reduced at higher salinities (e.g., 6 and 8 ppt); whereas *juvenile* channel catfish were unaffected at salinity concentrations as high as 10 ppt.
- With a 96-hr LC50 value of 3.8 ppt, *larval* fathead minnows (<24 hrs old) were adversely affected at salinities normally expected in Salt Creek at moderately low flows. Survival in the 3.4 ppt ambient Salt Creek sample was 70 percent.
- The health of the juvenile fathead minnows and channel catfish used in the Salt Creek in-situ program appears to be acceptable based on laboratory reference toxicant testing.
- Acute and chronic effluent toxicity testing of Theresa Street and Northeast WWTP effluents was conducted twice during the in-situ program.
- Chronic toxicity testing was conducted exposing *larval Ceriodaphnia dubia* and fathead minnows to Little Salt Creek water. The IC25 value for the minnows was >100 percent creek water, but the IC25 value for *C. dubia* was 27 percent Little Salt Creek water.
- Results from analyses of pesticides and herbicides in samples collected from Salt Creek did not indicate detected concentrations which explain the toxicological results from the in-situ program.

**Electronic Version:** [Go to Manuscript 5](#) – Data Integration and Site-Specific Criteria Compliance (next manuscript)

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[Links to Supporting Data Files](#)

**TABLE 4-1 SUMMARY OF RESULTS OF 15-DAY FATHEAD MINNOW SALINITY/  
GROWTH STUDY**

Test Species: *Pimephales promelas*  
 Test: 15-day Survival and Growth test  
 Client Name: City of Lincoln  
 Sample Description: Synthetic Salt Creek  
 EA QC Test Number: TN-99-585

Test Concentration (ppt Synthetic Salt Creek)	7-Day Survival (percent)	15-Day Survival (percent)	Mean Biomass (mg/exposed organism $\pm$ S.D.)
Control	100	100	16.0 ( $\pm$ 0.40)
2	100	97	15.2 ( $\pm$ 1.27)
4	100	100	13.5 ( $\pm$ 0.61) <sup>(a)</sup>
6	97	97	13.5 ( $\pm$ 0.83) <sup>(a)</sup>
8	97	97	12.1 ( $\pm$ 1.04) <sup>(a)</sup>

Mean initial dry weight ( $\pm$  S.D.) = 9.46 ( $\pm$ 0.40)

Endpoints (expressed as ppt Synthetic Salt Creek)

48-Hour LC50:	>8
NOEC:	2
LOEC:	4
ChV:	2.8
IC20:	7

<u>Selected Test Water Quality Parameters</u>	<u>Range</u>	<u>Mean (<math>\pm</math>S.D.)</u>
Temperature ( $^{\circ}$ C):	23.9 – 25.1	24.4 ( $\pm$ 0.48)
pH:	7.5 – 8.8	8.3 ( $\pm$ 0.42)
Dissolved Oxygen (mg/L):	5.1 – 8.0	7.2 ( $\pm$ 0.96)
Salinity (ppt):	0.2 – 8.2	4.0 ( $\pm$ 2.81)

<sup>(a)</sup> Significantly different than the control (p = 0.05).

**TABLE 4-2 SUMMARY OF RESULTS OF 30-DAY FATHEAD MINNOW SALINITY/ GROWTH STUDY.**

Test Species: *Pimephales promelas*  
 Test: 30-day Survival and Growth test  
 Client Name: City of Lincoln  
 Sample Description: Synthetic Salt Creek

EA QC Test Number: TN-99-586

Test Concentration (ppt Synthetic Salt Creek)	15-Day Survival (percent)	30-Day Survival (percent)	Mean Biomass (mg/exposed organism $\pm$ S.D.)
Control	97	87	23.67 ( $\pm$ 0.742)
2	97	97	23.01 ( $\pm$ 0.148)
4	97	87	21.7 ( $\pm$ 2.008)
6	100	0 <sup>(a)</sup>	- -
8	97	0	- -

Mean Initial dry weight ( $\pm$ S.D) = 9.46 ( $\pm$ 0.40)

Endpoints (expressed as ppt Synthetic Salt Creek)

48-Hour LC50: >8  
 NOEC: 4  
 LOEC: 6  
 ChV: 4.9  
 IC20: >4  
 IC25: >4

Selected Test Water Quality Parameters	Range	Mean ( $\pm$ S.D.)
Temperature ( $^{\circ}$ C):	24.0 – 25.0	24.5 ( $\pm$ 0.35)
pH:	7.7 – 8.7	8.3 ( $\pm$ 0.34)
Dissolved Oxygen (mg/L):	6.5 – 8.1	7.6 ( $\pm$ 0.59)
Salinity (ppt):	0.2 – 8.5	4.2 ( $\pm$ 3.02)

<sup>(a)</sup> Significant mortality on Day 19 due to toxic batch of synthetic Salt Creek. Same batch of water did not similarly affect *I. Punctatus*. Data not included in Inhibition Concentration analyses.

**TABLE 4-3 SUMMARY OF RESULTS OF 30-DAY CHANNEL CATFISH SALINITY/  
GROWTH STUDY.**

Test Species: *Ictalurus punctatus*  
 Test: 30-day Survival and Growth test  
 Client Name: City of Lincoln  
 Sample Description: Synthetic Salt Creek

EA QC Test Number: TN-99-587

<u>Test Concentration</u> <u>(ppt Synthetic Salt Creek)</u>	<u>15-Day Survival</u> <u>(percent)</u>	<u>30-Day Survival</u> <u>(percent)</u>	<u>Mean Biomass</u> <u>(mg/exposed organism (±S.D.))</u>
2	100	100	199.9 (±23.3)
4	100	100	218.7 (±3.87)
6	100	100	214.9 (±15.3)
8	100	100	187.0 (±21.2)
10	100	100	185.6 (±47.5)

Mean Initial dry weight (±S.D.) = 120.3 (±16.5)

Endpoints (expressed as ppt Synthetic Salt Creek)

48-Hour LC50:	>10
NOEC:	10
LOEC:	>10
ChV:	>10
IC20:	>10
IC25:	>10

<u>Selected Test Water Quality Parameters</u>	<u>Range</u>	<u>Mean (±S.D.)</u>
Temperature (°C):	23.8 – 25.4	24.6 (±0.61)
pH:	7.9 – 8.8	8.3 (±0.38)
Dissolved Oxygen (mg/L):	4.3 – 7.8	6.3 (±1.50)
Salinity (ppt):	2.0 – 10.7	6.1 (±2.99)



**TABLE 4-4 SUMMARY OF RESULTS OF PIMEPHALES PROMELAS 96-HOUR ACUTE TOXICITY TEST WITH SYNTHETIC SALT CREEK WATER**

Test Organism: *Pimephales promelas* (<24 hours old)  
Sample Description: Synthetic Salt Creek, and ambient Salt Creek  
Test Date: 19-23 October 1999  
EA QC Test Number: TN-99-749

Test Concentration (ppt Salinity)	96-Hour Percent Survival
Control	100
1.8	85
2.8	60
4.2	50
6.5	55
10	10
Salt Creek (3.4 ppt)	70
48-Hour LC50:	3.8 (3.0 – 5.0) <sup>(a)</sup> ppt salinity

<u>Selected Test Water Quality Parameters</u>	<u>Range</u>	<u>Mean (±SD)</u>
Temperature (°C)	24.1 – 26.0	25 (±0.4)
pH	7.5 – 8.7	8.2 (±0.3)
Dissolved oxygen (mg/L)	7.2 – 8.4	7.8 (±0.4)
Salinity (ppt)	0.2 – 10.3	4.5 (±3.0)

<u>Sample Water Quality Parameters at Receipt</u>	<u>AT9-1477 (Salt Creek)</u>
pH	8.5
Dissolved Oxygen (mg/L)	7.7
Salinity (ppt)	3.4
Total Residual Chlorine (mg/L)	n/a

<sup>(a)</sup> Values in parentheses are 95 percent confidence limits.

**TABLE 4-5 SUMMARY OF ICTALURUS PUNCTATUS REFERENCE TOXICANT TEST**

Test Organism: *Ictalurus punctatus*  
Sample Description: Sodium Dodecyl Sulfate (SDS)  
EA QC Test Number: RT-99-162

<u>Test Concentration</u> <u>(mg/L SDS)</u>	<u>48-Hour</u> <u>Percent Survival</u>
Control	100
5	100
10	100
20	90
40	0
60	0

48-Hour LC50 (mg/L SDS): 26.2 (20 – 40) <sup>(a)</sup>

<u>Selected Test Water Quality Parameters</u>	<u>Range</u>	<u>Mean (±SD)</u>
Temperature (°C)	19.3 - 20.7	20.0 (±0.56)
pH	7.2 - 8.2	7.8 (±0.36)
Dissolved oxygen (mg/L)	2.1 - 9.5	6.6 (±2.36)
Conductivity (µmhos)	310 - 351	331 (±15.9)

<sup>(a)</sup> Values in parentheses are 95 percent confidence limits.

**TABLE 4-6 SUMMARY OF RESULTS OF PIMEPHALES PROMELAS REFERENCE TOXICANT TEST**

Test Organism: *Pimephales promelas*  
 Sample Description: Sodium Dodecyl Sulfate (SDS)  
 EA QC Test Number: RT-99-163

<u>Test Concentration</u> <u>(mg/L SDS)</u>	<u>24-Hour</u> <u>Percent Survival</u>
Control	100
5	100
10	100
20	100
40	0
60	0

48-Hour LC50 (mg/L SDS): 28.3 (20 – 40)<sup>(a)</sup>

<u>Selected Test Water Quality Parameters</u>	<u>Range</u>	<u>Mean (±SD)</u>
Temperature (°C)	19.5 – 19.7	19.6 (±0.05)
pH	7.5 – 8.1	7.9 (±0.21)
Dissolved oxygen (mg/L)	5.3 – 9.2	7.8 (±1.51)
Conductivity (µmhos)	268-331	321 (±16.9)

<sup>(a)</sup> Values in parentheses are 95 percent confidence limits.

**TABLE 4-7 RESULTS OF ACUTE TOXICITY TESTS CONDUCTED ON SAMPLES FROM THE CITY OF LINCOLN**

Sample Description:	Theresa Street WWTP		Northeast WWTP	
EA Accession Number:	AT9-1353		AT9-1352	
EA Test Number:	TN-99-672	TN-99-671	TN-99-674	TN-99-673
Test Species:	<i>C. dubia</i>	<i>P. promelas</i>	<i>C. dubia</i>	<i>P. promelas</i>
Test Concentration (percent effluent)	24-Hour Percent Survival	24-Hour Percent Survival	24-Hour Percent Survival	24-Hour Percent Survival
Control	95	100	100	100
Whole Effluent				
12.5	100	100	95	100
25	100	100	100	90
50	5	45	100	75
80	0	5	100	60
100	0	0	100	10
<b>24-Hour LC50 (percent effluent):</b>	<b>37.0 (25-50)<sup>(a)</sup></b>	<b>49.2 (41.5-55.7)<sup>(a)</sup></b>	<b>&gt;100</b>	<b>83.3 (72.7-88.8)<sup>(a)</sup></b>
Ammonia Stripped				
80	5	95	100	100
100	0	95	100	100
<b>24-Hour LC50 (percent effluent):</b>	<b>&lt;80</b>	<b>&gt;100</b>	<b>&gt;100</b>	<b>&gt;100</b>
Ammonia Addback				
80	0	0	90	0
100	0	0	0	0
<b>24-Hour LC50 (percent effluent):</b>	<b>&lt;80</b>	<b>&lt;80</b>	<b>87.3 (80-100)<sup>(a)</sup></b>	<b>&lt;80</b>

(a) Values in parentheses represent 95 percent confidence limits.

**TABLE 4-8 RESULTS OF CHRONIC TOXICITY TESTING ON SAMPLES FROM THE CITY OF LINCOLN - THERESA STREET WWTP (SEPTEMBER 1999)**

Sample Description:	Theresa Street WWTP				Theresa Street WWTP			
Sample Dates:	26-27 SEP, 28-29 SEP, 30 SEP-1 OCT 99				26-27 SEP, 28-29 SEP, 30 SEP-1 OCT 99			
EA Accession Numbers:	AT9-1353, AT9-1362, AT9-1370				AT9-1353, AT9-1362, AT9-1370			
EA Test Number:	TN-99-675				TN-99-677			
Test Species:	<i>C. dubia</i>				<i>P. promelas</i>			
Test Concentration (percent effluent)	48-Hour % Survival	96-Hour % Survival	7-Day % Survival	Mean Young Production (neonates/organism±SD)	48-Hour % Survival	96-Hour % Survival	7-Day % Survival	Mean Dry Weight (mg/organism±SD)
Control	90	80	80	28.1 (±15.6)	97	97	93	0.503 (±0.054)
Whole Effluent								
12.5	100	100	100	39.5 (±11.6)	98	98	98	0.454 (±0.037)
25	100	100	100	37.3 (±8.6)	100	93	90	0.461 (±0.046)
50	0	0	0 <sup>(a)</sup>	0 <sup>(a)</sup>	68	48	38 <sup>(b)</sup>	0.126 (±0.071) <sup>(a)</sup>
80	0	0	0 <sup>(a)</sup>	0 <sup>(a)</sup>	18	0	0 <sup>(a)</sup>	--
100	0	0	0 <sup>(a)</sup>	0 <sup>(a)</sup>	0	0	0 <sup>(a)</sup>	--
<u>Endpoints as percent effluent</u>								
48-Hour LC50 <sup>(c)</sup> :	35.4 (25-50)				58.6 (51.3-64.7)			
96-Hour LC50 <sup>(c)</sup> :	35.4 (25-50)				48.4 (41.2-65.2)			
NOEC:	25				25			
LOEC:	50				50			
ChV:	35.4				35.4			
IC25 <sup>(c)</sup> :	31.3 (28.7-31.3)				30.7 (21.7-36.4)			

(a) A concentration which has no surviving organisms or significant mortality is not included in statistical analyses.

(b) Concentration was significantly different from the control.

(c) Values in parenthesis represent 95 percent confidence limits.

NC=95 percent confidence limits not calculable.

**TABLE 4-8 (Continued)**

Sample Description:	Theresa Street WWTP				Theresa Street WWTP			
Sample Dates:	26-27 SEP, 28-29 SEP, 30 SEP-1 OCT 99				26-27 SEP, 28-29 SEP, 30 SEP-1 OCT 99			
EA Accession Numbers:	AT9-1353, AT9-1362, AT9-1370				AT9-1353, AT9-1362, AT9-1370			
EA Test Number:	TN-99-676				TN-99-678			
Test Species:	<i>C. dubia</i>				<i>P. promelas</i>			
Test Concentration (percent effluent)	48-Hour % Survival	96-Hour % Survival	7-Day % Survival	Mean Young Production (neonates/organism±SD)	48-Hour % Survival	96-Hour % Survival	7-Day % Survival	Mean Dry Weight (mg/organism±SD)
Control	100	90	90	24.2 (±11.7)	100	100	100	0.423 (±0.034)
Ammonia Stripped								
80	100	100	90	21.4 (±10.0)	95	93	93	0.421 (±0.063)
100	70	70	60	11.9 (±11.0) <sup>(b)</sup>	100	100	100	0.433 (±0.038)
<u>Endpoints as percent effluent</u>								
48-Hour LC50 <sup>(c)</sup> :			>100				>100	
96-Hour LC50 <sup>(c)</sup> :			>100				>100	
NOEC:			80				100	
LOEC:			100				>100	
ChV:			89.4				>100	
IC25 <sup>(c)</sup> :			86.8 (NC)				>100	

(a) A concentration which has no surviving organisms or significant mortality is not included in statistical analyses.

(b) Concentration was significantly different from the control.

(c) Values in parenthesis represent 95 percent confidence limits.

NC=95 percent confidence limits not calculable.

**TABLE 4-9 RESULTS OF CHRONIC TOXICITY TESTING ON SAMPLES FROM THE CITY OF LINCOLN - NORTHEAST WWTP (SEPTEMBER 1999)**

Sample Description:	Northeast WWTP				Northeast WWTP			
Sample Dates:	26-27 SEP, 28-29 SEP, 30 SEP-1 OCT 99				26-27 SEP, 28-29 SEP, 30 SEP-1 OCT 99			
EA Accession Numbers:	AT9-1352, AT9-1361, AT9-1371				AT9-1352, AT9-1361, AT9-1371			
EA Test Number:	TN-99-679				TN-99-681			
Test Species:	<i>C. dubia</i>				<i>P. promelas</i>			
Test Concentration (percent effluent)	48-Hour % Survival	96-Hour % Survival	7-Day % Survival	Mean Young Production (neonates/organism±SD)	48-Hour % Survival	96-Hour % Survival	7-Day % Survival	Mean Dry Weight (mg/organism±SD)
Control	100	89	89	23.8 (±10.1)	100	100	98	0.454 (±0.058)
Whole Effluent								
12.5	100	90	90	30.0 (±12.0)	98	95	95	0.443 (±0.011)
25	100	90	90	28.7 (±11.7)	93	93	93	0.434 (±0.049)
50	100	100	90	12.5 (±7.6) <sup>(b)</sup>	74	69	69 <sup>(b)</sup>	0.535 (±0.095) <sup>(c)</sup>
80	100	90	90	0.8 (±1.6) <sup>(b)</sup>	23	3	3 <sup>(b)</sup>	0.210 (±0) <sup>(c)</sup>
100	100	50	20 <sup>(b)</sup>	0 <sup>(c)</sup>	3	0	0 <sup>(c)</sup>	--
<u>Endpoints as percent effluent</u>								
48-Hour LC50 <sup>(d)</sup> :			>100				62.5 (55.3-70.9)	
96-Hour LC50 <sup>(d)</sup> :			100				56.1 (50.8-60.6)	
NOEC:			25				25	
LOEC:			50				50	
ChV:			35.4				35.4	
IC25 <sup>(d)</sup> :			36.4 (23.8-40.1)				55.1 (22.4-59.1)	

(a) Control survival was below the minimum acceptable limit of 80 percent.

(b) Concentration was significantly different from the control.

(c) A concentration which has no surviving organisms or significant mortality is not included in statistical analyses.

(d) Values in parenthesis represent 95 percent confidence limits.

NC=95 percent confidence limits not calculable.

**TABLE 4-9 (Continued)**

Sample Description:		Northeast WWTP			Northeast WWTP			
Sample Dates:		26-27 SEP, 28-29 SEP, 30 SEP-1 OCT 99			26-27 SEP, 28-29 SEP, 30 SEP-1 OCT 99			
EA Accession Numbers:		AT9-1352, AT9-1361, AT9-1371			AT9-1352, AT9-1361, AT9-1371			
EA Test Number:		TN-99-680			TN-99-682			
Test Species:		<i>C. dubia</i>			<i>P. promelas</i>			
Test Concentration (percent effluent)	48-Hour % Survival	96-Hour % Survival	7-Day % Survival	Mean Young Production (neonates/organism±SD)	48-Hour % Survival	96-Hour % Survival	7-Day % Survival	Mean Dry Weight (mg/organism±SD)
Control	100	80	78 <sup>(a)</sup>	20.9 (±11.4)	100	100	95	0.454 (±0.024)
Ammonia Stripped								
80	100	90	60	22.2 (±12.5)	95	95	90	0.466 (±0.075)
100	100	100	100	21.9 (±9.9)	98	90	88	0.466 (±0.059)
<u>Endpoints as percent effluent</u>								
48-Hour LC50 <sup>(d)</sup> :			>100				>100	
96-Hour LC50 <sup>(d)</sup> :			>100				>100	
NOEC:			100				100	
LOEC:			>100				>100	
ChV:			>100				>100	
IC25 <sup>(d)</sup> :			>100				>100	

(a) Control survival was below the minimum acceptable limit of 80 percent.

(c) Concentration was significantly different from the control.

(b) A concentration which has no surviving organisms or significant mortality is not included in statistical analyses.

(d) Values in parenthesis represent 95 percent confidence limits.

NC=95 percent confidence limits not calculable.



**TABLE 4-10 RESULTS OF ACUTE TOXICITY TESTS CONDUCTED ON SAMPLES FROM THE CITY OF LINCOLN**

Sample Description:	Theresa Street WWTP		Northeast WWTP	
Sample Date:	10-11 October 1999		10-11 October 1999	
EA Accession Number:	AT9-1424		AT9-1423	
EA Test Number:	TN-99-725	TN-99-726	TN-99-727	TN-99-728
Test Species:	<i>C. dubia</i>	<i>P. promelas</i>	<i>C. dubia</i>	<i>P. promelas</i>
Test Concentration (percent effluent)	<u>24-Hour Percent Survival</u>	<u>24-Hour Percent Survival</u>	<u>24-Hour Percent Survival</u>	<u>24-Hour Percent Survival</u>
Control	100	100	100	90
Whole Effluent				
12.5	100	100	100	100
25	100	100	100	95
50	100	85	100	100
80	100	0	100	5
100	100	0	85	0
<b>24-Hour LC50 (percent effluent):</b>	<b>&gt;100</b>	<b>59.0 (50-80)<sup>(a)</sup></b>	<b>&gt;100</b>	<b>65.2 (50-80)<sup>(a)</sup></b>
Ammonia Stripped				
80	100	100	100	95
100	100	100	100	100
<b>24-Hour LC50 (percent effluent):</b>	<b>&gt;100</b>	<b>&gt;100</b>	<b>&gt;100</b>	<b>&gt;100</b>
Ammonia Addback				
80	40	0	40	0
100	0	0	5	0
<b>24-Hour LC50 (percent effluent):</b>	<b>&lt;80</b>	<b>&lt;80</b>	<b>&lt;80</b>	<b>&lt;80</b>

(a) Values in parentheses represent 95 percent confidence limits.

**TABLE 4-11 RESULTS OF CHRONIC TOXICITY TESTING ON SAMPLES FROM THE CITY OF LINCOLN - THERESA STREET WWTP (OCTOBER 1999)**

Sample Description:	Theresa Street WWTP				Theresa Street WWTP			
Sample Dates:	10-11, 12-13, 14-15 October 1999				12-13, 14-15, 17-18 October 1999			
EA Accession Numbers:	AT9-1424, AT9-1459, AT9-1465				AT9-1459, AT9-1465, AT9-1476			
EA Test Number:	TN-99-729				TN-99-742			
Test Species:	<i>C. dubia</i>				<i>P. promelas</i>			
Test Concentration (percent effluent)	48-Hour % Survival	96-Hour % Survival	8-Day % Survival	Mean Young Production (neonates/organism±SD)	48-Hour % Survival	96-Hour % Survival	7-Day % Survival	Mean Dry Weight (mg/organism±SD)
Control	100	100	90	17.2 (±9.1)	100	100	100	0.682 (±0.033)
Whole Effluent								
12.5	100	100	90	40.0 (±14.5)	100	100	98	0.705 (±0.018)
25	100	100	100	49.8 (±9.6)	100	100	100	0.621 (±0.023) <sup>(a)</sup>
50	100	100	30 <sup>(a)</sup>	8.2 (±5.1) <sup>(b)</sup>	50	25	25 <sup>(a)</sup>	0.758 (±0.071) <sup>(b)</sup>
80	80	40	0 <sup>(b)</sup>	0 <sup>(b)</sup>	0	0	0 <sup>(b)</sup>	--
100	100	0	0 <sup>(b)</sup>	0 <sup>(b)</sup>	0	0	0 <sup>(b)</sup>	--
<u>Endpoints as percent effluent</u>								
48-Hour LC50 <sup>(c)</sup> :	>100				50			
96-Hour LC50 <sup>(c)</sup> :	75.4 (50-100)				41.6 (25-50)			
NOEC:	25				12.5			
LOEC:	50				25			
ChV:	35.4				17.7			
IC25 <sup>(c)</sup> :	33.1 (32.5-34.2)				31.2 (27.7-34.5)			

(a) Concentration was significantly different from the control.

(b) A concentration which has no surviving organisms or significant mortality is not included in statistical analyses.

(c) Values in parenthesis represent 95 percent confidence limits.

NC=95 percent confidence limits not calculable.

**TABLE 4-11 (Continued)**

Sample Description:		Theresa Street WWTP				Theresa Street WWTP			
Sample Dates:		10-11, 12-13, 14-15 October 1999				12-13, 14-15, 17-18 October 1999			
EA Accession Numbers:		AT9-1424, AT9-1459, AT9-1465				AT9-1459, AT9-1465, AT9-1476			
EA Test Number:		TN-99-730				TN-99-743			
Test Species:		<i>C. dubia</i>				<i>P. promelas</i>			
Test Concentration (percent effluent)	48-Hour % Survival	96-Hour % Survival	6-Day % Survival	Mean Young Production (neonates/organism±SD)	48-Hour % Survival	96-Hour % Survival	7-Day % Survival	Mean Dry Weight (mg/organism±SD)	
Control	100	100	100	19.1 (±2.7)	100	100	100	0.696 (±0.028)	
Ammonia Stripped									
80	90	90	20 <sup>(a)</sup>	23.5 (±8.7) <sup>(b)</sup>	100	100	100	0.706 (±0.066)	
100	100	100	10 <sup>(a)</sup>	19.0 (±5.4) <sup>(b)</sup>	100	100	100	0.695 (±0.095)	
<u>Endpoints as percent effluent</u>									
48-Hour LC50 <sup>(c)</sup> :			>100		>100				
96-Hour LC50 <sup>(c)</sup> :			>100		>100				
NOEC:			<80		100				
LOEC:			80		>100				
ChV:			<80		>100				
IC25 <sup>(c)</sup> :			>100		>100				

(a) Concentration was significantly different from the control.

(b) A concentration which has no surviving organisms or significant mortality is not included in statistical analyses.

(c) Values in parenthesis represent 95 percent confidence limits.

NC=95 percent confidence limits not calculable.

**TABLE 4-12 RESULTS OF CHRONIC TOXICITY TESTING ON SAMPLES FROM THE CITY OF LINCOLN - NORTHEAST WWTP (OCTOBER 1999)**

Sample Description:		Northeast WWTP				Northeast WWTP			
Sample Dates:		10-11, 12-13, 14-15 October 1999				12-13, 14-15, 17-18 October 1999			
EA Accession Numbers:		AT9-1423, AT9-1458, AT9-1466				AT9-1458, AT9-1466, AT9-1475			
EA Test Number:		TN-99-731				TN-99-740			
Test Species:		<i>C. dubia</i>				<i>P. promelas</i>			
Test Concentration (percent effluent)	48-Hour % Survival	96-Hour % Survival	6-Day % Survival	Mean Young Production (neonates/organism±SD)	48-Hour % Survival	96-Hour % Survival	7-Day % Survival	Mean Dry Weight (mg/organism±SD)	
Control	100	100	90	19.7 (±7.3)	100	100	100	0.650 (±0.091)	
Whole Effluent									
12.5	100	100	100	27.3 (±4.1)	100	98	98	0.734 (±0.029)	
25	100	90	90	23.2 (±9.1)	100	98	98	0.672 (±0.060)	
50	100	100	100	17.1 (±2.9)	95	80	75 <sup>(a)</sup>	0.673 (±0.068) <sup>(b)</sup>	
80	100	100	100	7.8 (±4.8) <sup>(a)</sup>	18	3	3 <sup>(a)</sup>	0.580 (±0.000) <sup>(b)</sup>	
100	90	80	70	0.2 (±0.4) <sup>(a)</sup>	0	0	0 <sup>(b)</sup>	--	
<u>Endpoints as percent effluent</u>									
48-Hour LC50 <sup>(c)</sup> :			>100				67.1 (62.3-71.4)		
96-Hour LC50 <sup>(c)</sup> :			>100				58.8 (50-80)		
NOEC:			50				25		
LOEC:			80				50		
ChV:			63.2				35.4		
IC25 <sup>(c)</sup> :			47.8 (24.8-53.8)				48.5 (39.1-54.2)		

(a) Concentration was significantly different from the control.

(b) A concentration which has no surviving organisms or significant mortality is not included in statistical analyses.

(c) Values in parenthesis represent 95 percent confidence limits.

NC=95 percent confidence limits not calculable.

**TABLE 4-12 (Continued)**

Sample Description:		Northeast WWTP			Northeast WWTP			
Sample Dates:		10-11, 12-13, 14-15 October 1999			12-13, 14-15, 17-18 October 1999			
EA Accession Numbers:		AT9-1423, AT9-1458, AT9-1466			AT9-1458, AT9-1466, AT9-1475			
EA Test Number:		TN-99-732			TN-99-741			
Test Species:		<i>C. dubia</i>			<i>P. promelas</i>			
Test Concentration (percent effluent)	48-Hour % Survival	96-Hour % Survival	6-Day % Survival	Mean Young Production (neonates/organism±SD)	48-Hour % Survival	96-Hour % Survival	7-Day % Survival	Mean Dry Weight (mg/organism±SD)
Control	100	100	100	19.0 (±4.5)	100	100	100	0.588 (±0.065)
Ammonia Stripped								
80	100	100	90	15.1 (±5.0)	100	100	98	0.604 (±0.049)
100	90	90	80	11.5 (±6.2) <sup>(a)</sup>	100	100	98	0.653 (±0.045)
<u>Endpoints as percent effluent</u>								
48-Hour LC50 <sup>(c)</sup> :			>100				>100	
96-Hour LC50 <sup>(c)</sup> :			>100				>100	
NOEC:			80				100	
LOEC:			100				>100	
ChV:			89.4				>100	
IC25 <sup>(c)</sup> :			84.7 (NC)				>100	

(a) Concentration was significantly different from the control.

(b) A concentration which has no surviving organisms or significant mortality is not included in statistical analyses.

(c) Values in parenthesis represent 95 percent confidence limits.

NC=95 percent confidence limits not calculable.

**TABLE 4-13      SUMMARY OF RESULTS OF 30-DAY LARVAL FATHEAD MINNOW  
AMMONIA/GROWTH STUDY**

Test Species: *Pimephales promelas*  
 Test: 30-day Survival and Growth test  
 Client Name: City of Lincoln  
 Sample Description: Ammonium chloride  
 EA QC Test Number: TN-00-042

Test Nominal Concentration (mg/L NH <sub>3</sub> -N)	7-Day Survival (percent)	30-Day Survival (percent)	Mean Biomass (mg/exposed organism ±S.D.)
Control	98	88	8.53 (±1.05)
0.87	95	85	8.92 (±1.25)
1.8	90	90	9.93 (±0.60)
3.7	92	87	9.99 (±0.48)
7.5	95	80	8.50 (±2.47)
15.5	95	98	8.69 (±1.06)

Test Endpoints (Nominal mg/L NH<sub>3</sub>-N)

48-Hour LC50: >15.5  
 NOEC: 15.5  
 LOEC: >15.5  
 ChV: >15.5  
 IC20: >15.5

<u>Selected Test Water Quality Parameters</u>	<u>Range</u>	<u>Mean (±S.D.)</u>
Temperature (°C):	22.0 – 27.5 <sup>(a)</sup>	23.9 (±1.72)
pH:	7.0 – 8.6	7.8 (±0.49)
Dissolved Oxygen (mg/L):	2.8 – 8.8	6.9 (±1.85)
Salinity (ppt):	1.9 – 2.6	2.2 (±0.20)

<sup>(a)</sup> Temperature was outside the target range of 25±1°C at times during the test period.

**TABLE 4-14 SUMMARY OF AMMONIA MEASUREMENTS COMPLETED ON INDIVIDUAL TEST SOLUTIONS TEST NUMBER TN-00-042**

Test	New Test Solutions (mg/L NH <sub>3</sub> -N)	Old Test Solutions (mg/L NH <sub>3</sub> -N) <sup>(a)</sup>
<u>Nominal Concentration (mg/L NH<sub>3</sub>-N)</u>	<u>Mean (± S.D.)</u>	<u>Mean (± S.D.)</u>
Control	0.23 (±0.01)	0.24 (±0.11)
0.87	0.65 (±0.25)	0.81 (±0.31)
1.8	1.50 (±0.63)	--
3.7	2.72 (±0.89)	2.80 (±0.70)
7.5	5.74 (±1.93)	--
15.5	11.85 (±3.35)	11.46 (±2.53)

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<sup>(a)</sup> Ammonia samples of old test solutions were only collected on control, 0.87, 3.7, 15.5 mg/L test concentrations.

**TABLE 4-15      SUMMARY OF RESULTS OF 30-DAY JUVENILE FATHEAD MINNOW  
AMMONIA/GROWTH STUDY**

Test Species: *Pimephales promelas*  
 Test: 30-day Survival and Growth test  
 Client Name: City of Lincoln  
 Sample Description: Ammonium chloride  
 EA QC Test Number: TN-00-043

Test Nominal Concentration (mg/L NH <sub>3</sub> )	7-Day Survival (percent)	30-Day Survival (percent)	Mean Biomass (mg/exposed organism ±S.D.)
Control	100	97	29.15 (±3.69)
1.8	97	87	24.19 (±3.68)
3.7	97	97	31.65 (±4.60)
7.5	100	92	26.27 (±5.26)
15.5	97	92	27.05 (±5.19)
32	100	90	20.99 (±1.59) <sup>(a)</sup>

Test Endpoints (Nominal mg/L NH<sub>3</sub>-N)

48-Hour LC50:	>32
NOEC:	15.5
LOEC:	32
ChV:	22.3
IC20:	25.2

<u>Selected Test Water Quality Parameters</u>	<u>Range</u>	<u>Mean (±S.D.)</u>
Temperature (°C):	22.0 – 27.3 <sup>(b)</sup>	24.0 (±1.69)
pH:	7.0 – 8.6	7.8 (±0.40)
Dissolved Oxygen (mg/L):	3.5 – 8.8	6.5 (±1.82)
Salinity (ppt):	2.4 – 2.4	2.2 (±0.19)

<sup>(a)</sup> Significantly different than the control (p = 0.05).

<sup>(b)</sup> Temperature was outside the target range of 25±1°C at times during the test period.



**TABLE 4-16 SUMMARY OF AMMONIA MEASUREMENTS COMPLETED ON INDIVIDUAL TEST SOLUTIONS TEST NUMBER TN-00-043**

Test Nominal Concentration <u>(mg/L NH<sub>3</sub>-N)</u>	New Test Solutions (mg/L NH <sub>3</sub> -N)	Old Test Solutions (mg/L NH <sub>3</sub> -N) <sup>(a)</sup>
	<u>Mean (± S.D.)</u>	<u>Mean (± S.D.)</u>
Control	0.23 (±0.01)	0.34 (±0.13)
1.8	1.51 (±0.62)	1.66 (±0.60)
3.7	2.72 (±0.89)	--
7.5	5.82 (±1.94)	5.74 (±1.57)
15.5	11.84 (±1.57)	--
32	20.87 (±4.43)	20.51 (±3.05)

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<sup>(a)</sup> Ammonia samples of old test solutions were only collected on control, 0.87, 3.7, 15.5 mg/L test concentrations.

**TABLE 4-17 HERBICIDES AND PESTICIDES DETECTED (ug/L) IN WATER SAMPLES COLLECTED DURING THE SALT CREEK IN SITU STUDY**

Parameter	24 September 1999			5 October 1999			20 October 1999		
	C	D	I	C	D	I	C	D	I
EPA 507 Modified									
Atrazine (0.20)*	0.6	0.4	0.3	0.4	0.3	0.3	ND	ND	ND
Deethylatrazine (0.20)	0.4	ND	ND	0.2	ND	ND	ND	ND	ND
Herbicides									
Dalapon (10.0)	ND	2.6 j	3.6 j	ND	4.4 j	4.2 j	ND	2.5 j	3.3 j
Dicamba (1.0)	0.3 j	0.2 j	0.3 j	0.30 jb	ND	ND	ND	ND	ND
MCPP (500)	220 j	ND	ND	ND	ND	ND	ND	ND	ND
MCPA (500)	940 p	580 p	920 p	390 j	ND	ND	ND	ND	ND
PCP (10)	0.1 j	0.2 j	0.3 j	ND	0.10 j	0.10 j	ND	ND	ND
2,4,5,-TP (1.0)	ND	ND	ND	ND	0.20 j	ND	ND	ND	ND
Chlorinated Pesticides/PCBs									
alpha-BHC (0.050)	ND	ND	ND	ND	ND	ND	ND	0.0049 j	.0059 j
Heptachlor Epoxide (0.050)	ND	ND	ND	ND	ND	ND	0.016 j	0.009 j	0.0068 j
gamma-Chlordane (0.050)	ND	ND	ND	ND	ND	ND	ND	0.011 j	0.020 j

\* = detection limit

j = qualifier indicating estimated value